TESTING SOIL FOR MICRONUTRIENTS[1]

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Introduction

Soil testing for micronutrients is increasing throughout the United States. Many commercial fertilizer companies include these tests as a regular part of their service.

For any valid soil test there are four main features that need careful consideration. First, the sample must represent the area sampled and must have been treated in such a manner as to avoid contamination or drastic change in the status of the nutrient under consideration. Second, the conditions for extracting the nutrient must be such that the amount extracted is related to the available nutrient supply. Third, the nutrient in the extract must be determined quantitatively and fourth, interpretation of the results must be based on reliable calibration data. In addition to these criteria, before a soil test is accepted and used widely, it must be reduced to a routine operation at moderate cost.

Detailed discussions of the chemistry of the micronutrients in the soil are given by Mitchell (19) and Hodgson (14). Comprehensive discussions dealing with various aspects of soil testing are available in a recent monograph (11) which includes a discussion by Viets (34) on soil testing for the micronutrient cations. Many of the wet chemistry procedures (3) for determining the micronutrients are time consuming and require highly trained personnel for accurate analysis. The advent of atomic absorption spectrophotometry, however, has eliminated many of the analytical problems and offers straightforward procedures that are free from interferences and may be used routinely.

Chemistry of Micronutrient Cations in Soil

The chemistry of micronutrients in soil is not well understood. On the basis of present knowledge, however, they are considered to be present in one or more of the following forms:

1. Soluble ions or complexes
2. Ions associated with mineral or organic surfaces
3. Major or minor components of precipitates
4. Major or minor components of primary and secondary minerals
5. Components of biological systems and their residues.

Zinc and copper are considered to be present in the +2 oxidation state. In this form they can be present in any of the five situations listed above. Iron and manganese, however, enter into oxidation-reduction reactions wherein they have more

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than one oxidation state. Iron is present as the ferrous (+2) or ferric (+3) forms, whereas manganese in soils is believed to have oxidation states of +2, +3 or +4. The amount of iron or manganese that is present in the different oxidation states may be altered drastically and sometimes rapidly by changing environmental conditions. Thus, low pH, low oxygen, and high organic matter favor the reduced forms of these elements. Conversely, the more oxidized states are favored in well-aerated mineral soils having pH's above 5.

All four elements considered here form very insoluble compounds with other soil constituents over the pH range of most soils. The reduced forms of iron and manganese are more soluble than the oxidized forms. All four elements are capable of forming soluble complexes with some of the organic compounds present in soils, thus increasing their solubilities at a given pH.

Plants absorb readily the soluble, exchangeable, and some of the organic complexed forms of micronutrients in soils. Nutrients contained in precipitates, at least those present on or near the surfaces of the particles are also readily taken up, but part of the nutrient may be occluded within the particles and hence unavailable as long as the particles remain intact. The release of micronutrients from secondary and primary minerals is very slow and, consequently, contributes little to the pool of available nutrients. Likewise, some of the micronutrients contained within soil organisms and their residues may turn over slowly and thus supply very little available nutrient to plants. The soil system containing all forms of the nutrients is not static; indeed it is very dynamic, with equilibria established among several of the different forms. To illustrate this principle, consider what happens when soluble forms of iron and zinc are added to calcareous soils. Immediately upon application and mixing with moist soil the soluble forms of these elements are increased. With time, however, the supply of soluble forms decreases as they react with other soil constituents and revert to less soluble forms. Because of the different chemical properties of these two elements, iron reverts more rapidly and more completely than does zinc. The supply of available zinc is increased for several years, although a large portion of that added soon reverts to unavailable forms. Consequently, application of zinc fertilizer corrects zinc deficiency for several years whereas soluble iron results in only a short-lived correction of iron chlorosis.

Because the micronutrient cations are held very tenaciously by the soil particles, and because of their slight solubilities over the pH range of most agricultural soils, they are relatively immobile in all but acid soils. Therefore, under most conditions the availabilities are higher in the surface layer of soil than in the subsoil. This results from enrichment of the surface soil from decaying vegetation on the soil surface during the long periods of soil formation or it may result from previous fertilizer applications. The consequence of this immobility is that plant roots must be near the source of available nutrient in order for uptake to occur. This does not mean that no movement occurs from the source, but simply that movement is very slight in comparison to a mobile nutrient such as nitrate-nitrogen. Thus, in a single growing season, probably only a small fraction of the total available micronutrient is taken up because of limited soil exploration by the crop's roots.

When these factors are considered, it is perhaps unreasonable to try to devise a soil test wherein the nutrient extracted is equal to that taken up by the crop. A soil test may reflect the availability of a nutrient throughout the whole soil mass, but a crop reflects the nutrient availability in only a small portion of the soil mass. Thus, the amount of nutrient extracted in a soil test procedure should be related to the amount taken up by the crop, but the amounts would not likely be in a 1:1 ratio. It is possible that plant roots may increase the

availability of an otherwise unavailable nutrient near their surfaces through chemical effects resulting from physiological processes occurring within the plant or on the root surfaces.

The chemical properties of the nutrient element and the soil are extremely important factors to consider when selecting an extracting solution for soil testing purposes. Because the element may be in several different forms, each of which may have different availabilities, the properties of the extracting solution should be such that the readily available forms are dissolved and the unavailable forms remain in the soil. Because of the limited solubility of the nutrients over the PH range 5 to 9, the extracting solution must be capable of holding the amounts of nutrients present in the soil sample. This may be accomplished by adjusting the soil: solution ratio, controlling the pH of the solution in contact with the soil, or adding chelating agents. For example, when hydrochloric acid is added to a calcareous soil suspended in water, very little zinc enters the liquid phase until enough acid is added to dissolve the carbonates present in the soil and to lower the pH of the system below 5.5 (21). This results from the limited solubility of zinc at higher pH's. Thus, very little zinc is removed from neutral or calcareous soils by water or neutral salt solutions. Copper, iron, and manganese act in the same manner, but their solubility-pH relationships differ from that of zinc.

In recent years chelating agents have been used for extracting micronutrients from soils. These compounds form stable complexes with metal-cations; the stabilities of the complexes vary with the different cations. The tendency for the micronutrient cations to form chelates with most chelating agents are many orders of magnitude greater than for calcium or magnesium. Thus, chelating agents form soluble complexes with small quantities of the micronutrients even in the presence of much larger amounts of calcium or magnesium. It is this selectivity along with the chelate's ability to support much larger concentrations of the micronutrient cations over the pH range 5 to 9 than is possible without the chelate that makes them useful in soil testing. This concept holds only within limits, but soil testing for micronutrients is ideally suited for its application because only small amounts (usually a few ppm) of nutrient are encountered in most soils.

In contrast to the example cited above where hydrochloric acid is added to a suspension of calcareous soil, if a chelating agent is added instead of the acid, zinc will appear in the liquid phase very rapidly, even though the pH remains near 8. This is because the chelate forms a strong soluble complex with the zinc. Thus, significant amounts of zinc may be extracted from soil evan at high pH's by use of these extracting agents. Again, copper, iron and manganese will be extracted in the same manner, but their tendencies to form chelates differ.

Crop Response As Related to Availability Indexes

Interpretation of soil test results is an extremely critical operation in the soil testing procedure. The question to be answered for micronutrients is that of "yes" or "no"; i.e., is fertilization needed or not? The answer given may have large financial impact on the grower. If the wrong answer is given, he may suffer a poor crop or he may have spent money for fertilizer needlessly. Either way he loses. On the other hand, if the soil test properly indicates the nutrient status of the soil, large financial returns may be realized through high-yielding crops of high quality or through having saved the cost of fertilizer. Thus, proper interpretation must be based on well-established relationships between plant growth and soil test levels obtained under various soil and cropping conditions.

A summary of published information dealing with soil test calibrations for zinc, copper, iron, and manganese follows. Only those situations where soil test results were related to the incidence of deficiency symptoms or growth responses to applied nutrient are included.

Zinc

Several attempts have been made to determine the soluble and exchangeable zinc in soils by extracting with neutral salt solutions. Potassium chloride (13), ammonium acetate (25, 33), and magnesium sulfate (18) solutions remove only traces of zinc from most soils. The values obtained by these methods do not appear to be related to zinc availability.

Extraction of soil with acid has been used by many for assessing the zinc status of soils. Wear and Sommer (36) devised a procedure using 0.1 N HCl as the extracting solution and compared the results obtained on 15 acid soils (pH 4.7 to pH 6.2) from Southeastern United States with those obtained using 0.04 N acetic acid. Eight of the samples were from areas growing zinc-deficient corn. Both methods gave results which correlated well with the incidence of zinc-deficiency symptoms, although greater amounts of zinc were extracted by 0.1 N HCl than by 0.04 N acetic acid. Values greater than 1.0 ppm zinc extracted by 0.1 N HCl and 0.5 ppm zinc extracted by 0.04 N acetic acid were considered adequate for normal crop growth.

The data of Nelson et al (21) indicate that for 26 noncalcareous soils included in their study, 11 of 16 soils containing less than 2 ppm acid-extractable zinc produced zinc-deficient plants, whereas 8 of 10 soils containing more than this amount produced healthy plants. Jackson et al (15) indicate that acid-extractable zinc levels greater than 3.0 ppm are required for normal growth of corn on acid soils of the Willamette Valley.

Acid extraction of zinc appears to be a reliable indicator for predicting zinc fertilizer needs on acid soils, but does not work well on calcareous soils. Acid-extractable zinc values for calcareous soils may be 6 ppm or more and still support zinc-deficient crops. Consequently, Nelson et al (21) included a separate analysis for titratable alkalinity along with the acid-extractable zinc for assessing the zinc status of neutral and calcareous soils. The method showed good results for all 51 soils tested. The higher the titratable alkalinity, the higher the acid-extractable zinc content must be to support normal crop growth. Presumably the titratable alkalinity is a correction factor which accounts for the unavailable forms of zinc dissolved by the acid. Painter et al (24) used the method for assessing the zinc status of calcareous soils in Wyoming. Soil samples were taken from 74 fields cropped to corn. Zinc-deficiency symptoms were evident on the corn in 51 of the 74 fields. The results of the survey indicated a clear separation of deficient and nondeficient fields when the criteria reported by Nelson et al (21) were applied to the data. Attempts at local calibration, however, were unsuccessful. In greenhouse studies, Trierweiler and Lindsay (32) reported poor separation of deficient and nondeficient soils by this method. In particular, the method failed to segregate soils where high levels of applied P induced zinc deficiency.

Shaw and Dean (25) proposed the use of dithizone (diphenylthiocarbazone)-extractable zinc for assessing the zinc status of soils. The use of this procedure involves a two-phase liquid system of aqueous normal ammonium acetate and dithizone in carbon tetrachloride. The zinc-dithizone complex accumulates in the organic phase during extraction because of its greater solubility in this medium. The mild action of the dithizone and its low concentration precludes dissolution of solid phase soil constituents such as phosphates, carbonates, or silicates. Under the

conditions of the extraction, however, several other heavy metals are also extracted. Values obtained by this method for 44 soils from many parts of the United States indicate that 1 ppm or more of dithizone-extractable zinc is required to support healthy plants; 17 of 24 soils testing less than 1.0 ppm dithizone-extractable zinc supported zinc-deficient plants, whereas 15 of 20 soils testing more than 1.0 ppm supported normal plants. The data were not segregated according to crop, a factor which may have improved the correlation because of the different zinc requirements of crops.

Viets et al (35), reporting on field experiments with corn in the Columbia Basin of Washington, indicated that where zinc-deficiency symptoms occurred, the soil contained less than 0.41 ppm dithizone-extractable zinc, but that other experiments were conducted wherein no zinc-deficiency symptoms occurred and the dithizone-extractable zinc was less than this value.

Brown et al (6) correlated the dithizone-extractable zinc with the response of sweet corn grown in the greenhouse to zinc applied to 53 California soils. Eighty-four percent of the soils having less than 0.55 ppm dithizone-extractable zinc responded to zinc fertilization, whereas 76 percent of those having values above this level supported normal plants.

Trierweiler and Lindsay (32) conducted similar tests with corn grown in the greenhouse on 42 Colorado soils. A clear-cut separation between deficient and nondeficient soils was obtained at a dithizone-extractable zinc level of 0.95 ppm. The value of 0.55 ppm used by Brown et al (6) when applied to the Colorado data clearly separated the deficient and nondeficient soils, but on several soils where P induced zinc deficiency the values were between 0.55 and 0.95 ppm dithizone-extractable zinc.

In their comprehensive comparisons of several methods for extracting zinc from soils, Trierweiler and Lindsay developed a new method using a mixture of 0.01 M EDTA and 1 M ammonium carbonate, pH 8.6, as the extracting solution. They related the zinc extracted by this solution to the incidence of zinc deficiency of corn grown in the greenhouse on 42 Colorado soils. A critical value of 1.4 ppm extractable zinc clearly separated responsive and nonresponsive soils, including those where zinc deficiency was induced by P applications.

Copper

In contrast to the great amount of work reported for zinc, little information of the same kind has been reported for copper. Several reports indicate that the critical levels for the various soil tests were exceeded for all soils tested since no deficiency symptoms or growth responses were obtained. Consequently, the soil test results were correlated with copper concentration or copper uptake. Such data are given by Blevins and Massey (4) for 34 Kentucky soils.

The soil tests proposed to date for copper do not seem to take into account some of the factors that affect the uptake of copper by plants grown under some conditions. Extractable aluminum interferes with copper uptake by millet (4). Likewise, Leibig et al (17) showed that aluminum moderated the toxic effects of copper on citrus grown in solution culture. The mechanism for this effect is not known. Much of the copper in soils has been shown to be associated with the organic fraction (16, 31). Thus, successful soil tests for copper should reflect the influences of these factors on copper availability to plants.

The various extracting solutions used to evaluate the copper status of soils are water (10), neutral salts (9), acidified salts (9, 10), acids (8, 9, 29), EDTA (4, 8, 9), and dithizone (4). Water and neutral salts extract very small quantities of copper and therefore are used only for special purposes. Fiskell (9) reports the sufficiency range for copper extracted by normal ammonium acetate (pH 4.0) to be 0.2 to 5.0 ppm. Above this range, toxicity is likely and below this range, deficiency is expected. Much higher values are obtained by using 0.1 N HCl as the extracting solution. This method readily reflects additions of copper to soils and, consequently, may be used to delineate areas where toxic amounts of copper have accumulated. Spencer (28) developed a test for doing this.

EDTA-extractable copper was reported by Cheng and Bray (8) to be similar to the amounts extracted by 0.1 N HCl. The values obtained, however, by either method were not related to copper deficiency of plants. Mitchell (20) reports the critical level for EDTA-extractable copper to be about 1 ppm for several crops grown in Scotland. Blevins and Massey (4) indicate, however, that millet did not show typical copper deficiency symptoms when grown in the greenhouse on soil containing 0.2 ppm EDTA-extractable copper, although some abnormalities were noted on plants grown on soils low in copper and/or high in extractable aluminum. Similar results are reported by these workers using ammonium acetate-dithizone as the extracting agent.

Manganese

Because the availability of manganese depends strongly on the redox potential of the soil and because the redox conditions can change rapidly, the time of sampling and the storage conditions for the samples must be closely controlled. For example, if soil in the field has been extremely wet for a long time in the presence of fresh organic material, the equilibria will strongly favor the presence of divalent manganese. If samples are taken at this time and analyzed directly, the exchangeable and under some conditions even the water-soluble manganese will be high. On the other hand if the samples are allowed to dry while exposed to the air, the redox conditions will change and much of the reduced manganese will revert to higher oxidation states and be precipitated as oxides. Analysis of the samples at this time may show low values for exchangeable manganese and likely no water-soluble manganese, but easily reducible manganese will likely be high. Thus, time of sampling and treatment of samples before analysis become important factors in assessing the manganese status of soils and even in choosing the method of analysis.

Manganese immediately available to plants is present in the soil as soluble and exchangeable divalent ions. According to Adams (1) deficiency levels of manganese are likely when the soil pH is above 6; toxicity levels are likely below pH 5. Thus, soil tests for this element should indicate toxic as well as deficient levels.

Water-soluble manganese (27) may be a useful diagnostic test, especially for toxic amounts under acid or high organic matter conditions. Under more alkaline conditions, however, the values obtained by this method may be essentially zero and hence reveal little about the manganese status of many agricultural soils.

A more useful test is that for exchangeable manganese. Normal solutions of ammonium acetate, calcium nitrate, and magnesium nitrate are commonly used for extracting exchangeable manganese. Beckwith (2) however, shows several advantages to extracting the exchangeable manganese with a mixture of ammonium acetate and disodium calcium EDTA. This reagent, because of its ability to complex and solubilize divalent manganese at high pH, distinguishes readily between exchangeable manganese and the precipitated oxides present in the soil.

Sherman (26) reports for Kentucky soils that satisfactory crop growth requires 3 ppm exchangeable manganese. Heintze (12) reports the same critical level in England, but mentions that in some cases healthy crops grow at lower levels. A value of 4 ppm exchangeable manganese adequately separates deficient and nondeficient soils in Denmark (5). Of the 39 soils tested, 95 percent containing less than this amount supported manganese-deficient crops, whereas 83 percent of the soils containing more than the critical value supported healthy plant growth. Time of sampling was shown to affect the values obtained because of fluctuating reducing conditions resulting from changing moisture contents during the season. Different species and varieties differed in their susceptibilities to manganese deficiency and toxicity.

Because of the complexity of manganese equilibria in soil, it is important to estimate the supply of this element that may become available with time and as conditions change. Thus, Sherman et al (27) devised a soil test which measures the easily reducible manganese. The manganese present in this form (various oxides of manganese in the +3 and +4 oxidation states) is a measure of the soil's ability to maintain an adequate supply of available manganese. The extracting solution used in this procedure is normal ammonium acetate containing 0.2 percent hydroquinone (a weak reducing agent). These workers report that manganese-deficient soils of Kentucky contain only trace amounts of water-soluble manganese and 2 to 5 ppm of exchangeable manganese. Consequently, easily reducible manganese is used as the criterion for assessing the manganese status of these soils. According to their data, soils containing less than 25 ppm of easily reducible manganese are unable to furnish adequate manganese for healthy plant growth. Productive soils usually contain 100 ppm or more of manganese subject to reduction.

Iron

Despite the widespread occurrence of iron chlorosis, soil tests for available iron have been unsuccessful in delineating deficient and nondeficient soils. Iron solubility in well-aerated mineral soils is very low because the major portion of the soil iron is in the +3 oxidation state. Hence its solubility is controlled by pH, aeration, and the presence of organic chelating compounds. Much of the work done on iron nutrition has been aimed at determining the effects of soil constituents such as calcium carbonate and excesses of copper and manganese on iron availability and the incidence of iron chlorosis. Plant physiological processes also play important roles in the iron chlorosis problem because of the great differences in susceptibilities to iron deficiency shown by different plant species and even different varieties within the same species. These factors are discussed in detail by Brown (7).

Olson (22) reviewed the status of soil tests for available iron and presents methods for its determination. Water-soluble iron is essentially zero for most soils and is not useful as a soil test for available iron.

Olson and Carlson (23) showed that less iron was extracted from soils supporting chlorotic sorghum plants by normal ammonium acetate adjusted to pH 4.8 than from soils where only trees were chlorotic or where no chlorosis was noted. The method has not been used widely, although modifications of it have been tried by others.

Thorne and Wallace (30) extracted easily reducible iron from soils by using normal ammonium acetate (pH 5.0) containing 0.2 percent hydroquinone. On paired samples from 14 sites, soils supporting chlorotic peaches, pears, and grapes at each site contained less readily reducible iron than did soils supporting healthy trees. There was much overlapping of values between sites, however. The values ranged from 0.3 to 10.0 ppm for soils supporting chlorotic plants and from 1.5 to

16.9 ppm for soils supporting healthy plants. Consequently, the method has not proved useful for delineating iron-deficient soils.

Summary

Soil testing is a reliable indicator for delineating conditions of deficiency and toxicity for some of the micronutrients. The data presented here indicate that soil testing for zinc is a practical means for predicting zinc fertilizer needs under many conditions. Acid-extractable zinc is reliable for assessing the zinc status of acid soils. It does not work well, however, on calcareous soils, unless titratable alkalinity is used to correct for the large amounts of unavailable zinc solubilized by the acid Because of their unique properties, the use of chelates appears to offer a simple, straightforward means for assessing zinc availability in all kinds of soils.

Much additional work is needed on soil testing for copper in the United States. Extraction with acidified salt solutions appears to be a reliable method and the use of chelates shows promise, but more work is needed relating soil test levels to the incidence of copper deficiency. Acid-extractable copper reliably indicates areas of copper toxicity.

Exchangeable and/or easily reducible manganese are reliable criteria for assessing deficiency and toxicity levels of this element. Data are lacking, however, for many areas.

Very little success has been attained in developing soil tests for available iron.

In view of the different critical levels reported for the same tests conducted under different soil, environmental, and cropping conditions, local calibration is recommended for any of the tests. Many factors affect plant growth that do not affect the levels of extractable nutrients. Additional improvements in soil testing will come as problems such as interactions between nutrients, the effects of organic matter, environmental conditions affecting growth and nutrient uptake, and the effects that plants themselves may have on nutrient availability are delineated and better understood.

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